

NAGESH PASUPALA, MS., Ph.D.
Scientist, Molecular and Cell Biology

QUALIFICATIONS

- 17 years of experience working in a laboratory setting; with hands-on lab bench work with various mammalian cell cultures (mouse embryonic cell lines, human cancer cell lines, T- cell lines, primary cell lines, etc), *Saccharomyces cerevisiae*, Bacterial strains and Viruses.
- Innovative, enthusiastic, self-motivated, highly organized and self-supervising team member.
- Worked in BSL2 laboratory settings at Johns Hopkins University and Uniformed Services University of the Health Sciences.
- More than 15 years of hands-on experience on wide variety of Molecular and Cell Biology, Genetics, Microbiology and Biochemistry procedures and techniques which include, but not limited to:
 - Protein, DNA and RNA isolation and purification from wide variety of model systems including mammalian cell culture, animal tissues, Yeast and Bacteria model systems.
 - Protein, DNA and RNA Gel Electrophoresis, transfer and blotting techniques (Western, Southern, Northern and slot blotting techniques).
 - Various Recombinant DNA technologies including (PCR, gene cloning in variety of plasmid and viral vectors using various techniques like traditional restriction enzyme based, ligation independent methods like Gibson assembly, site-directed mutagenesis, etc)
 - Genetic engineering of various mammalian cell lines, human cancer cell lines and T- cell lines using CRISPR-Cas9, CRISPR-RNP, Adeno and Lenti-viral vectors, PiggyBac transposon system.
 - Gene knockout, knockdown and tagging in various mammalian and human cell cultures using plasmid, siRNA, shRNA transfection.
 - Gene silencing and epigenetics studies by Chromatin Immunoprecipitation Assays (ChIP), RT-PCR, qPCR, Real-Time PCR.
 - Transcription and translation studies by mRNA quantification using quantitative RT-PCR, cDNA library preparation, Cycloheximide chase experiments. In vitro and in vivo post-translational assays to study protein modifications by SUMO and Ubiquitin.
 - Experience working with radioactive phosphorus -32 isotope containing nucleotide to generate DNA probes for southern blotting.
 - Epigenetics and gene expression studies by extracting R-loops (RNA-DNA hybrids) from HTLV-1 virus and *H. pylori* bacteria infected human suspension and adherent cell lines, genomic library and R-loop library construction for sequencing on Illumina.
 - Bioinformatic analysis of genomic library sequence using Python.
 - Confocal and Immunofluorescence microscopy imaging of mammalian and yeast cells.
 - Flow cytometry sorting of fluorescent labelled cells.
 - Experience working with DNA and RNA viruses, Viral vector constructions, Viral Titration-Plaque assays, Viral vector transduction in mammalian and human cell lines.
- Advance knowledge on statistical analysis and plotting charts using PRISM software. Advance experience using Adobe Photoshop for making publishable quality figures, Experience using MS office (Word, Excel, Power point).

EDUCATION

University of Hyderabad
Ph.D in Molecular Genetics
Awards/Honors:
July 2005 to June 2010: CSIR Research Fellowship.

Hyderabad, India
July 2005 to Oct 2011

University of Hyderabad

Hyderabad, India

Master's in Biotechnology

July 2002 to May 2004

Awards/Honors:

Department of Biotechnology (DBT), Govt of India Fellowship for development of Master's research.

SKD University

Bachelor's in Biochemistry

Ananthapur, India

July 1999 to June 2002

PROFESSIONAL EXPERIENCE

**Uniformed Services University of the Health Sciences
Through Henry M Jackson Foundation for Military Medicine
Bethesda, MD- 20814-4799**

Oct 2018 - Present

Research Scientist -1

1. Studying the molecular mechanisms of adult T cell leukemia development in HTLV-1 virus infected individuals.
2. Studying prognosis of gastric cancer in *H. pylori* infected human gastric epithelial cells.
3. Conducted mammalian and human T cell cultures, Viral vector transductions, cell viability assays, viral titration determination by Plaque assays, Plasmid transfections in various mammalian cell lines. Limiting dilution and clonal expansion of stable cell lines.
4. Generated gene knock down and knockout using siRNA, shRNA and CRISPR-Cas9 system.
5. Isolated Protein from mammalian cell cultures, animal tissue samples and bacterial cells. Sub-cellular fractionation of mammalian cell lysates. Conducted Co-IPs, Western blotting, Slot blotting.
6. Isolated DNA and RNA from human T cells, cancer cell lines and mouse stomach tissue. Constructed genomic libraries and R-loop (RNA-DNA hybrid) libraries for sequencing on illumina. Bioinformatic analysis of sequence data.
7. Isolation and culturing of CD4+ and CD25+ T cells from PBMC samples.
8. Flow cytometry sorting of fluorescent labelled cells.
9. Responsible for analyzing and interpreting results, presenting research data in department-wide and university-wide conferences and seminars, generate data for grant proposals, writing scientific reports, develops and executes new protocols, maintains research lab operations and equipments, ordering and receiving lab reagents and supplies.

**Johns Hopkins University/School of Medicine
Baltimore, MD-21205**

Mar 2015 – Sept 2018

Postdoctoral Research Fellow

1. Investigated the molecular mechanism of Otub1 enzyme mediated Ubiquitin signaling in DNA Damage repair and cancer.
2. Conducted mammalian cell culture experiments. Genetically engineered human cancer cell lines using CRISPR-Cas9 techniques, Transfected mammalian cell cultures with plasmids, siRNA, shRNA using lipofectamine, Calphos and electroporation methods.
3. Isolated DNA and RNA from mammalian cell lines, constructed cDNA library, performed quantitative RT-PCR to analyze gene expression and epigenetics.
4. Isolated protein from mammalian cell cultures by Co-IPs and performed Western blotting. Sub-cellular fractionation of mammalian cell lysates. Performed in vitro biochemical analysis of protein functions, Performed in vitro and in vivo protein ubiquitination assays.
5. Performed protein expression and purification by affinity chromatography methods from mammalian, yeast and bacterial cell cultures.

6. Cloned several human and yeast genes in various plasmid vectors using traditional restriction enzyme and ligation based, ligation independent methods like PCR and Gipson assembly, Cre-recombinase based gateway cloning, site-directed mutagenesis, etc
7. Responsible for maintaining detailed records of experiments and their results and prepare the information resulting from these experiments for presentations at laboratory-wide, University-wide meetings and international publications.

**College of William and Mary
Williamsburg, VA**

Feb 2012 – Feb 2015

Postdoctoral Fellow

1. Conducted experiments to identify novel protein substrates of SUMO targeted Ubiquitin ligase enzymes in *Saccharomyces cerevisiae*
2. Investigated the molecular mechanism of SUMO and Ubiquitin mediated elimination of aggregated Human Huntington protein in human cell line and yeast model system.
3. Protein purification and quantitation from yeast cell cultures, Co-IPs, Protein gel electrophoresis on native and gradient polyacrylamide gels, Protein transfer and Immunoblotting methods.
4. DNA isolation, PCR based cloning, large scale genome insertion and deletion in yeast cells by various recombination methods. Cloned several genes in various plasmid vectors using traditional restriction enzyme and ligation based, ligation independent methods like PCR and Gipson assembly, Cre-recombinase based gateway cloning, site-directed mutagenesis, etc
5. Conducted protein-protein interaction by yeast two hybrid methods.
6. Responsible for general lab management, consumable orders, maintenance of equipment, maintaining laboratory supplies and equipment including chemicals, biological materials, etc.
7. Supervised, mentored several undergraduate students on various molecular biology techniques.
8. Responsible for maintaining detailed records of experiments and their results and prepare the information resulting from these experiments at department-wide and national conferences and publications.

**University of Hyderabad
Hyderabad, Andhra Pradesh, India**

July 2005 - Oct 2011

Graduate Student

1. Conducted genetic screen in *Saccharomyces cerevisiae*. Constructed genomic library and several yeast strains with gene modifications and knockouts.
2. Performed Chromatin Immunoprecipitation (Ch-IP) of telomere regions in yeast cells.
3. Performed quantitative and real-time PCR of the Ch-IP extracted DNA.
4. Cloned several genes in various plasmid vectors using traditional restriction enzyme and ligation based, ligation independent methods like PCR and Gipson assembly, Cre-recombinase based gateway cloning, site-directed mutagenesis, etc
5. Extracted Protein from yeast cells by Co-IPs and conducted Protein gel electrophoresis and Western blotting to analyze protein modifications.
6. Isolated total cellular DNA and RNA. Performed Southern and Northern blotting assays to study the telomere length under various genetic and epigenetic conditions.
7. Used Radioactive phosphorus -32 nucleotides to generate DNA fragments for probing yeast telomeres in southern blots.
8. Supervised and mentored several Master students to complete their thesis work.
9. Provided specialized scientific support, including literature review for new methods and procedures on the design, planning, conduct, and publication of experiments.

LABORATORY SKILLS AND TECHNIQUES

- Mammalian cell culture, primary cell culture, yeast molecular genetics.
- Immunoprecipitation, protein expression, purification and characterization.
- Gel Electrophoresis (SDS-PAGE), Native gel, Western blotting, slot and dot blotting techniques.
- Co-IPs, Chromatin Immunoprecipitation (Ch-IPs), R-loops (RNA-DNA hybrid) capture.
- DNA, RNA isolation, cDNA library preparation from mammalian cell cultures, yeast cells and animal tissue samples.
- PCR, qPCR, RT-PCR and real-time PCR.
- Plasmid and viral (Adeno, Lenti-) vector construction, Cloning techniques (PCR, Gibson assembly, Cre/LoxP recombinase, gateway cloning)
- DNA and RNA transfection in mammalian cells.
- CRISPR-Cas9 and gRNA based gene knockout in mammalian cell lines and Human T cells.
- Genomic library and R-loop library construction for sequencing on illumina platforms and Bioinformatic analysis of sequence data.
- Microscopy (light, fluorescence and confocal microscopy) imaging of mammalian and yeast cells.
- Affinity chromatography for protein purification
- Mammalian cell transfection, transduction and electroporation
- Protein quantification (Bradford, BCA assays)
- Gene silencing, transcriptomic and proteomic analysis
- Flow cytometry sorting of fluorescent labeled cells.

PUBLICATIONS:

1. **Nagesh Pasupala**, Chou Zen Giam and D. Scott Merrell. H. pylori infection enhances R-loop formation and induces DNA Double Strand Breaks in gastric adenocarcinoma cells. (manuscript under preparation)
2. Chou Zen Giam and **Nagesh Pasupala**. NF- κ B-Induced R-loops and Genomic instability in HTLV-I infected and Adult T-Cell Leukemia Cells. *Viruses*, 2022, 14 (5) 877.
3. Yunlong He*, **Nagesh Pasupala***, Huijun Zhi*, Imran Hussain, Hsiu-Ming Shih, Sharmistha Bhattacharyya, Roopa Biswas, Oliver John Semmes and Chou-Zen Giam. NF- κ B-Induced R-loop accumulation and DNA damage select for Nucleotide Excision Repair deficiencies in Adult T-cell Leukemia. *PNAS*, 2021.
(*joint first authors)
4. Huijun Zhi, Xin Guo, Yik-khuan Ho, **Nagesh Pasupala**, Hampus Engstrom, Oliver John Semmes and Chou-Zen Giam. RNF8 dysregulation and down-regulation during HTLV-1 infection promote genomic instability in Adult T-cell Leukemia. *PLoS Pathog*nes (2020). 16 (5), e1008618.
5. **Nagesh Pasupala***, Marie Morrow*, Lauren T. Que, Barbara Malynn, Averil Ma, Cynthia Wolberger. OTUB1 non-catalytically regulates the stability of the E2 ubiquitin conjugating enzyme UBCH6. *Journal of Biological Chemistry* (2018). 293, 18285-18295. (* joint first authors)
6. Kentaro Ohkuni*, **Nagesh Pasupala***, Jennifer Peek, Grace Lauren Holloway, Gloria Driessnack-Sciar,

Reuben Levy-Myers, Richard E. Baker, Munira A. Basrai and Oliver Kerscher. SUMO-targeted ubiquitin ligases (STUbLs) reduce the toxicity and abnormal transcriptional activity associated with a mutant, aggregation-prone fragment of huntingtin. *Front Genet.* 2018 Sep 18;9:379. doi: 10.3389/fgene.2018.00379. eCollection 2018 (* joint first authors)

7. Kentaro Ohkuni, Yoshimitsu Takahashi, Alyona Fulp, Josh Lawrimore, Wei-chun Au, **Nagesh Pasupala**, Ruben Levy-Mayers, Jack Warren, Alexander Strunnikov, Richard E. Baker, Oliver Kerscher, Kerry Bloom and Munira A. Basrai (2016). SUMO Targeted Ubiquitin Ligase (STUbL) Slx5 Regulates proteolysis of centromeric Histone H3 variant Cse4 and prevents its mislocalization to euchromatin. *Molecular Biology of the Cell*, Vol 27, 1500-1510.
8. Jason W. Westerbeck*, **Nagesh Pasupala***, Mark Guillotte, Eva Szymanski, Brooke C Matson, Ceceilia Esteban and Oliver Kerscher (2014). A SUMO-targeted ubiquitin ligase is involved in the degradation of the nuclear pool of the SUMO E3 ligase Siz1. *Molecular Biology of the Cell*, Vol 25, 1-16. (* joint first authors)
9. **Pasupala N**, Easwaran S, Hannan A, Shore D, Mishra K. (2012). The SUMO E3 ligase Siz2 exerts a locus-dependent effect on gene silencing in *Saccharomyces cerevisiae*. *Eukaryotic Cell*, Vol 11, 452-462.
10. M.Sritharan, S.Ramadevi, **N Pasupala**, S.Taine, S Asuthkar (2005). In silico identification and modeling of a putative iron-regulated TonB-dependent outer membrane receptor protein from the genome of *Leptospira interrogans* serovar Lai. *Online Journal of Bioinformatics* 6, 74-90.